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Predictive power of the second renal biopsy in lupus nephritis: Significance of macrophages

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Background. A new Biopsy Index containing the Glomerular Activity (GAI), Tubulointerstitial Activity (TIAI), Chronic Lesion (CLI), and Immunofluorescence (IFI) indices was developed, showing better correlations with clinical and outcome parameters than the National Institutes of Health Activity and Chronicity Indices (AI and CI) in lupus nephritis. This report examines the ability of these indices and individual morphologic variables to predict doubling of serum creatinine (S_{Cr}; CRX2).

Methods. Renal biopsies from 71 patients with lupus nephritis with an initial biopsy (Bx1) and systematic control biopsy (Bx2) after six months of therapy were studied. Kaplan–Meier survival curves were developed for each index and morphologic variable at each biopsy. A subset of 30 biopsies was stained with the macrophage marker PGM1.

Results. At Bx1, only the TIAI and the quantity of C3 and vascular staining on IF were predictive of CRX2. At Bx2, particularly predictive of CRX2 were the GAI, IFI, Biopsy Index, and BxInfl, a composite variable comprised of all of the inflammatory variables. Among individual variables, glomerular and tubular macrophages correlated the best with clinical and outcome parameters. Crescents and karyorrhexis/fibrinoid necrosis also correlated with outcome. Neither the NIH CI or our CLI, nor the TIAI correlated with outcome. In 30 biopsies stained with PGM1, PGM1+ cells correlated well with glomerular and tubular macrophages identified on routine stains and showed even better correlations with S_{Cr}, proteinuria, and progression to renal insufficiency than the latter. A diffuse membranoproliferative (MPGN) pattern was seen in seven patients at Bx1. In four of the seven patients, MPGN disappeared with therapy, and all finished with normal renal function. However, among the three patients in whom MPGN persisted and eight patients in whom MPGN, focal or diffuse, appeared under therapy, six reached end-stage renal disease, and a seventh died with marked renal insufficiency.

Conclusions. The biopsy index and its components correlate modestly with CRX2 at Bx1, but strongly at Bx2, particularly IFI, BxInfl, and glomerular and tubular macrophages. Stains for macrophage markers form a valuable adjunct in interpretation of renal biopsies in systemic lupus erythematosus (SLE). MPGN features do not have an ominous significance at Bx1, but their persistence or appearance under therapy are associated with poor outcome.

Key words: NIH Activity and Chronicity Indices, serum creatinine, membranoproliferative nephritis, chronic renal insufficiency, inflammation.

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We have had the opportunity to study a series of 71 patients with initial biopsies and systematic control biopsies at six months after the induction of therapy [1]. On the basis of this material, a new biopsy index was developed, composed of four elements: Glomerular Activity Index (GAI), a modification of the standard NIH Activity Index (AI) [2, 3] with the addition of glomerular monocytes and elimination of interstitial inflammation; Tubulointerstitial Activity Index (TIAI) analogous to the GAI, evaluating several tubular epithelial and inflammatory components, including interstitial inflammation, but excluding tubular atrophy; Chronic Lesions Index (CLI), a modification of the standard NIH CI [2, 3], with the addition of glomerular scars; Immunofluorescence Index (IFI), a semiquantitative index of immunofluorescence (IF) staining of glomerular capillary and mesangial, tubulointerstitial, and vascular elements by six standard antisera. This new biopsy index and/or its components showed better correlations with nearly all clinical and outcome parameters, including serum creatinine (S_{Cr}), proteinuria, doubling of S_{Cr} (CRX2), end-stage renal disease (ESRD), and final renal function than the standard AI and CI [1].

The purpose of the present communication is threefold. The first is to evaluate the power of the biopsy index and its component morphologic variables in predicting CRX2. There has been dissatisfaction with the presently employed AI and CI in terms of their predictive power, and Investigators seem to be in agreement that the AI seems to have little predictive power [4–9], at least at first biopsy. A number of investigators have found that the CI was moderately predictive of renal survival [2, 3,

7, 8, 10–14], although some have not [4, 6, 9, 15]. Other combinations of variables have been used in an attempt to improve the correlations between biopsy and outcome with some success [2, 11, 12], but none has been entirely satisfactory. Similarly, in our current study, at the time of the first biopsy very few variables were predictive of outcome, and none was predictive in a manner that could be applied to an individual case, because of the marked overlap between cases that did and did not progress to CRX2. However, at the time of the second biopsy, a number of features were predictive of CRX2 in a fashion that would permit the pathologist to indicate to the clinician that the patient was at risk of progression to CRX2, despite a relatively quiescent clinical picture.

Second, our previous communication revealed unexpectedly strong correlations between glomerular monocytes and tubular macrophages on the one hand and clinical and outcome parameters on the other [1]. To explore these findings in more detail, we used immunohistochemical staining of a subset of biopsies with the macrophage marker PGM1 (a monoclonal antibody specific for the CD68 cell surface antigen of macrophages).

Third, the significance of the membranoproliferative variant of diffuse proliferative lupus nephritis, characterized by the presence of capillary double contours, was explored since 19 of the 71 patients showed this pattern, focally or diffusely, at some point in their course.

METHODS

Patient population

Renal biopsies and clinical data from 71 patients from four Paris hospitals (Bichat, Broussais, Henri Mondor, and St. Louis) from the period 1986 to 1994 were evaluated. All patients had at least four ARA criteria, with positive antinuclear and/or anti-DNA preparations in all. The patients had the following demographic distribution: There were 63 females and 8 males, with a mean age of 36.8 ± 13.8 years at first biopsy; ethnic origins were 40 Caucasian, 13 North African, 10 Asiatic, and 8 black patients. Mean follow-up was 7.6 ± 2.9 years (range of 0.7 to 13.4 years). All patients who had an initial renal biopsy (Bx1), with systematic second biopsy (Bx2) at six months after induction treatment, were included. From the third biopsy onward, biopsies were performed primarily for clinical indications and are not discussed in detail here, but will be reported in a subsequent communication. All biopsies were reviewed by one pathologist (G.S.H.) who was blind to the clinical data. Cases were categorized according to the 1982 World Health Organization criteria [16]. No case of focal proliferative GN or of mixed membranous and focal proliferative GN was diagnosed with fewer than 10 glomeruli.

Technical methods and biopsy data

Specifics of technical methods are given in the prior communication [1]. Briefly, light microscopic slides were prepared and stained according to standard methods for light and IF microscopy. For technical reasons, six cases at Bx1 were unsatisfactory for detailed morphologic evaluation, although diagnosis of diffuse proliferative lupus nephritis was established on the basis of paraffin-embedded IF material. Photographs of positive IF were available in 122 (85.9%) of the 142 first and second biopsies, and the biopsy report systematically detailed all results. In the remaining cases, photographs were not available and/or the specimen consisted of medulla only.

The following clinical parameters were evaluated at the time of each biopsy: systemic lupus erythematosus activity index (SLEDAI) [17], blood pressure, CH50, C3, C4, anti-DNA antibodies (DNA), S_{Cr} (μmol/L), proteinuria (g/24 h), and hematuria (rbcs/mL), hemoglobin, and platelets. Clinical data were available for all patients for the initial two biopsies, but for six patients, follow-up data as to outcome are incomplete.

Four overlapping outcome parameters were measured: (*1*) doubling of the initial S_{Cr} (CRX2) for three months or more; (*2*) end-stage renal disease (ESRD), requiring dialysis and/or transplant; (*3*) renal relapse (Renrel), recrudescence of renal disease after an initial therapeutic response, as defined by a recent increase of S_{Cr} by >50% with active urinary sediment and/or increase in proteinuria to 3.5 g/day or greater; and (*4*) final renal function (RF_{last}), the last S_{Cr}, with an arbitrary value of 500 μmol/L assigned to all patients with ESRD, on dialysis or transplanted.

Morphologic variables

The schema for evaluation of morphologic variables has been reported in detail in our earlier communication [1]. Briefly, the method of grading morphologic lesions parallels that used by Austin et al [2, 3], but adds consideration of tubular lesions and IF data. As in their system, the various morphologic lesions were graded on a scale of 0 to 3+, with 1+ corresponding to involvement of less than 25% of the glomeruli or parenchyma by the variable in question; 2+, 25 to 50%; and 3+, greater than 50% of the glomeruli/parenchyma. We modified this grading system slightly, giving a value of 0.5+ to lesions that were present, but minimal, for example, less than 5% of glomeruli, in the hope of refining somewhat the distinctions in the many instances in which lesions of a given type were not widespread. The GAI, CI, and TIAI were formulated using this approach.

An IFI was devised in the following manner. Antisera to the following were employed in all cases: IgG, IgA, IgM, C3, C1q, and fibrinogen. Four separate morphologic components were evaluated: glomerular capillary

Table 1. Components of Biopsy Index

Index name	Symbol	Scale
Glomerular Activity Index (GAI)		
Glomerular proliferation	*glprolif*	0–3+
Polymorphonuclear leukocytes	*glpmn*	0–3+
Karyorrhexis/fibrinoid necrosis	*karyfib*	(0–3+) X2
Cellular crescents	*cresc*	(0–3+) X2
Hyaline deposits	*hyaldep*	0–3+
Glomerular monocytes	*glmono*	0–3+
Maximum:		24
Tubulointerstitial Activity Index (TIAI)		
Tubular cell pyknosis	*tubpyk*	0–3+
Tubular nuclear "activation"	*tubact*	0–3+
Tubular cell necrosis	*tubnec*	0–3+
Tubular cell flattening	*tubflat*	0–3+
Macrophages in tubular lumens	*macrlum*	0–3+
Epithelial cells in tubular lumens	*eplum*	0–3+
Interstitial inflammation	*intinfl*	0–3+
Maximum:		21
Chronic Lesions Index (CLI)		
Glomerulosclerosis	*glscl*	0–3+
Glomerular scars	*gloscar*	0–3+
Fibrous crescents	*fibcres*	0–3+
Tubular atrophy	*tubatro*	0–3+
Interstitial fibrosis	*intfib*	0–3+
Maximum:		15
Immunofluorescence Index (IFI)		
Glomerular capillary IF	*glcapif*	(0–4+) X6 antisera
Glomerular mesangial IF	*glmesif*	(0–4+) X6 antisera
Tubulointerstitial IF	*tubulif*	(0–4+) X6 antisera
Vascular IF	*vascif*	(0–4+) X6 antisera
Maximum:		96

$$\text{Biopsy Index} = \frac{\text{GAI}}{8} + \frac{\text{CI}}{5} + \frac{\text{TIAI}}{7} + \frac{\text{IF Index}}{32} = \text{Maximum 12.}$$

IF (*glcapif*), glomerular mesangial IF (*glmesif*), vascular IF (*vascif*), and tubulointerstitial IF (*tubulif*). The degree of staining for each antiserum was graded on a scale of 0 to 4+ for each component, and the totals for all the antisera were then added for a maximum of 24 for each component. In addition to the IFI, total staining for each antiserum was calculated (maximum for 16 per antiserum). The overall biopsy index, with the abbreviations used in this article, is shown in Table 1.

In addition, for this study, glomerular capillary deposits were divided by location on photographs of the IF. Three sorts of deposits were distinguished: The first were definite subendothelial deposits, recognizable by smooth outer contour, corresponding to the glomerular basement membrane and often encroaching on the capillary lumen. These were often fairly long, but occasionally short and somewhat comma shaped. The second were definite subepithelial deposits, discontinuous and granular in nature, on the external aspect of the basement membrane (large transmural deposits were sometimes recognized, and were included in the estimation of both subendothelial and subepithelial deposits), and third were deposits of indefinite location. These were usually small and/or pale, but occasionally deposits were included in this category because their location could not be determined because of the plane of section, overlap with other capillary loops, etc. We estimate this group to constitute roughly 30% of glomerular capillary deposits. Deposits in each category were graded semiquantitatively on a scale of 0 to 4+, relying primarily on the extent of deposits in the tuft.

Arterial and arteriolar lesions were recorded but were not included in the Biopsy Index because their correlations with clinical and outcome variables were relatively weak, and their inclusion in the biopsy index weakened its correlations. In the present study, only vascular IF was included.

Immunohistochemistry

Immunostaining for the macrophage marker PGM1 (Dakopatts, Trappes, France) was performed on a subset of 30 biopsies from the overall series, 14 from Bx1, and 16 from Bx2, as follows: Freshly cut paraffin slides were digested with pronase, followed by blocking with goat serum diluted 1/20 for 20 minutes. They were then incubated 30 minutes with PGM1, washed with Tris buffer, pH 7.4, followed by 30 minutes of incubation with horse anti-mouse antibody to detect the PGM1, washed, and then incubated with a preformed avidin-biotinylated alkaline phosphatase complex. Development with vector red AEC (3-amino-9-ethylcarbazol) substrate and counterstaining with hematoxylin completed the process.

Macrophage cell counts

Slides were examined using an ocular grid subdivided into 100 squares, yielding an overall area of approximately 10,000 μ^2 at $\times 40$ magnification. Only the cortical parenchyma was evaluated. For each biopsy, all glomeruli were evaluated, plus the equivalent of at least 100 grids of tubulointerstitium, with the exception of two biopsies in which only 72 and 80 grids could be counted because of limited material. The following morphologic compartments were evaluated: (*1*) glomerular capillary lumenal macrophages, (*2*) glomerular macrophages not within capillaries, (*3*) macrophages outside the glomerular tuft in Bowman's space, (*4*) tubular lumenal macrophages, (*5*) cells staining positively for PGM1 within the tubular epithelium, and (*6*) interstitial macrophages.

Statistical analyses

For continuous clinical variables (for example, S_{Cr}), Pearson product-moment correlation coefficients were calculated. The various morphologic indices, with maximal values ranging from 15 to 96, were treated as continuous variables. In comparing semiquantitative variables, such as hyaline deposits graded 0 to 3+, with continuous variables Pearson product-moment correlations were used. For comparisons of semiquantitative variables with one another, Spearman rank order correlations were used. For categorical variables (for example, presence or

absence of ESRD), the Spearman rank order correlation test was used. Since these correlations are very extensively cited, to maintain continuity in the text, they usually appear simply in parentheses, and asterisks are used to indicate the level of probability: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$. Thus, $r = 0.42$, $P = 0.0006$ would appear as (****0.42). Comparisons of survival were done by the Kaplan–Meier method, with differences in survival curves evaluated by log rank sum testing. Survival curves were calculated from the time of the first biopsy. Calculations were performed using Statistica® Version 5 (Statsoft, Inc., Tulsa, OK, USA).

RESULTS

Patient material and initial treatment

Initial diagnoses in this series were diffuse proliferative (WHO Class IV) lupus nephritis, 55 patients; focal proliferative (WHO Class III) lupus nephritis, 9 patients; and mixed membranous and focal proliferative (1982 WHO Class Vc) lupus nephritis, 7 patients. Fifty-eight of the 71 patients had newly diagnosed renal involvement; 13 patients had previously been treated for renal involvement with a variety of regimens. These latter patients did not differ from other patients in level of morphologic lesions or clinical data at the time of entry to this study. As might be anticipated, these patients tended to do somewhat worse as a group than the other patients, with a rate of CRX2 of 46.1 versus 23.1% for the remaining patients, but this difference did not reach the level of significance ($P = 0.10$). In addition, 15 patients had been previously treated for extrarenal manifestations, generally with low dose steroids and/or chloroquine. They even more nearly approached the remaining patients in terms of morphologic lesions, clinical data, and rates of CRX2 (33.3 vs. 26%, $P = 0.57$).

The six-month induction treatment consisted of monthly intravenous cyclophosphamide combined with prednisone (0.9 ± 0.4 mg/kg body weight/day for one month tapered to 0.4 ± 0.1 mg/kg body weight/day at 6 months) in 58 patients and corticosteroids alone in 13 patients (1.4 ± 0.3 mg/kg body weight/day tapered to 0.5 ± 0.16 mg/kg body weight/day at 6 months). Initial treatment was followed at six months by re-evaluation and control renal biopsy to evaluate the effects of therapy. Therapy was adjusted at this point by the clinician in the light of the clinical setting and biopsy findings. (In general, in the face of biopsies showing continuing activity, treatment was more aggressive than in patients with more quiescent biopsies, but treatment was not completely standardized, and evaluation of that treatment is outside the scope of this article.) Subsequent biopsies were carried out primarily for clinical indications. There was no difference in clinical data at entry or in outcome between those treated with cyclophosphamide plus steroids and those treated by steroids alone.

Eighteen patients eventually doubled their S_{Cr} (CRX2), all of whom had diffuse proliferative (WHO Class IV) lupus nephritis at initial biopsy. None reached CRX2 during the initial six months of treatment. A point to be stressed, there were no significant differences in any of the laboratory parameters at Bx2 between those who later progressed to CRX2 and those who did not. Differences in degree of hematuria approached significance (37 ± 63 vs. 14 ± 33 k rbc/mL, $P = 0.068$), but the wide range of values rendered this useless in distinguishing between the groups. Twelve patients ended in ESRD, with dialysis and/or transplantation, and five patients died, of whom three had arrived at CRX2. All patients with focal proliferative lesions finished with S_{Cr} at or near normal. One patient with mixed membranous and proliferative lesions died rapidly of septicemia with $S_{Cr} = 350$ μmol/L (but before technically having doubled her creatinine for the requisite 3 months). The other patients with this lesion finished with normal function or mild renal insufficiency.

Overall morphologic changes between biopsy 1 and biopsy 2

The alterations in the various morphologic parameters between Bx1 and Bx2 will be dealt with extensively in a future communication (manuscript in preparation), but certain generalizations must be made in order to put the present survival data into context. Taken globally, the data provide evidence that the most immediate effect of therapy is reduction of the inflammatory components, such as crescents and interstitial inflammation, with lesser reductions in the amount of immune deposits by IF. The second generalization that can be made is that, although initial levels were similar in the two groups, reductions between biopsies were invariably less in the group subsequently developing CRX2 (CRX2 = 1). As an example, values for the GAI for the group not doubling their S_{Cr} (CRX2 = 0) are 10.4 ± 4.9 declining to 2.9 ± 2.9 at Bx2. For the CRX2 = 1 group, the GAI began at comparable levels, 11.2 ± 4.9 but declined only to 6.1 ± 4.7 at Bx2. Thus, although there is no difference between initial values in the two groups, the difference between the two at Bx2 is substantial ($P = 0.0021$).

Taking these processes collectively and indexing initial values for the CRX2 = 0 group to 1 at Bx1 to facilitate comparison, overall immune deposits by IF declined from 1.0 to 0.5426 in the CRX2 = 0 group. In the CRX2 group, they started slightly (but not significantly) higher, 1.1131 but descended only to 0.8622 for CRX2 = 1, the difference between the two groups at Bx2 being highly significant ($P = 0.0035$). Similarly, BxInfl, a composite variable composed of all of the inflammatory morphologic variables, declined from 1.0 to 0.2176 for CRX = 0; comparative values for the CRX2 = 1 group being 1.2222 descending

to 0.6384 for CRX2 = 1 ($P < 0.00005$). There were fluctuations among individual inflammatory variables and in immune deposits according to whether they were considered by location (for example, subendothelial, tubulointerstitial) or by antiserum type (for example, anti-IgG), but all followed these general trends. By contrast, tubular epithelial alterations and chronic lesions such as interstitial fibrosis rose (chronic lesions) or fell (tubular epithelial lesions) in parallel in the two groups, the differences not reaching statistical significance.

Prediction of outcome

The predictive power of each of the morphologic variables and morphologic indices in terms of survival from developing CRX2 up to 10 years was evaluated at both Bx1 and Bx2 by standard Kaplan–Meier survival curves, with the significance of differences between curves determined by log rank sum testing. All patients were considered in these calculations (although, in fact, in this series, only those with diffuse proliferative lesions reached CRX2).

At Bx1, neither the standard AI and CI nor the overall biopsy index was predictive of progression to CRX2, although the TIAI taken alone was weakly predictive (Table 2). The only individual morphologic variables reaching statistical significance were C3 on IF and vascular IF.

At the second biopsy, the biopsy index and two of its component indices, GAI and IFI (Fig. 1A), showed significant differences in survival from CRX2 divided simply above and below their means, with greater differences in survival with selection of values further from the mean. At Bx2, the standard AI showed significant differences divided above and below the mean, with greater differences with selection of appropriate values. In contrast, neither the standard CI nor our revised CLI nor the TIAI served to separate survivals from CRX2 adequately. Of the composite variables comprised of multiple individual variables, BxInfl, composed of all of the inflammatory morphologic variables (*glpmn* + *glmono* + *cresc* + *karyfib* + *intinfl* + *pmnlum* + *macrlum*), showed the greatest ultimate separation between cases. The mean value was 1.73, dividing cases into 83.5 versus 22.3% survivals at 10 years ($P = 0.00006$; Fig. 1B). However, selecting cases with values <1.0 and >4.0 provided CRX2 survivals of 94.4% at 4500 days versus 0% at 1707 days onward ($P = 0.00005$; Fig. 2).

At the second biopsy, the simple presence or absence of karyorrhexis/fibrinoid necrosis, cellular crescents, glomerular monocytes, and tubular macrophages had significant predictive power for CRX2. The strongest of these individual morphologic variables was tubular lumenal macrophages, with CRX2 survivals at 10 years of 73.9% for those without tubular macrophages versus 18.2% for those with ($P = 0.0072$; Fig. 3). Among the components of the IFI, glomerular capillary, tubulointerstitial, and vascular IF showed significant predictive value, but glomerular mesangial IF did not.

Monocytes and macrophages

Glomerular monocytes and tubular macrophages are worthy of separate comment. At Bx1, glomerular macrophages were the most sensitive among the glomerular variables [glomerular proliferation, polymorphonuclear cells (PMNs), karyorrhexis, crescents, hyaline deposits, and macrophages] in distinguishing among clinical parameters. Evaluating the correlations of these variables with CH50, DNA, S_{Cr}, proteinuria, hematuria, and hemoglobin (Hgb), glomerular macrophages had the highest mean coefficient of determination (r^2) at 0.0825 ± 0.0529. The other glomerular variables had r^2s ranging between 0.0177 ± 0.0295 ($P < 0.00001$) and 0.0588 ± 0.0636 ($P = 0.028$).

At Bx2, differences in clinical parameters were much more muted because of the decline in overall morphologic activity. However, now the simple presence or absence of glomerular monocytes became a significant prognostic factor for CRX2 (Table 2).

Similarly, tubular lumenal macrophages (*macrlum*) were the most powerful of any of the variables constituting the TIAI. The patients with *macrlum* ≥1 (13 patients) at Bx1 were manifestly sicker than those with *macrlum* = 0 (40 patients). They had a higher frequency of hypertension ($P = 0.022$). DNAs were higher ($P = 0.006$), and the S_{Cr} was nearly double that in patients without lumenal macrophages (190 ± 107 vs. 106 ± 53, $P = 0.0005$). At Bx2, the disparity in those with (14 patients) and those without (56 patients) tubular macrophages was similar, with higher DNAs ($P = 0.039$) and higher S_{Cr} ($P = 0.0005$). More importantly, as indicated previously in this article, the simple presence or absence of macrophages in the tubular lumens was the single best individual morphologic variable determining survival in terms of CRX2 (Table 2). Overall, among the tubular morphologic variables, tubular lumenal macrophages had the highest correlation with clinical parameters at Bx1 and the highest correlation with outcome parameters at Bx2 and the best combination of clinical and outcome correlations for the two biopsies considered together (data not shown).

Immunohistochemical studies

Macrophage staining for PGM1 revealed excellent correlations with the simple variables glomerular monocytes and tubular lumenal macrophages in the biopsy index (Table 3). These variables had been evaluated semiquantitatively 0 to 3+ on the basis of the percentage of glomeruli with recognizable monocytes in excess of one and the percentage of tubular profiles containing recognizable macrophages on routine stains. First, glomerular monocytes identified on routine stains correlated well with their closest counterpart, glomerular capillary $PGM1^+$ cells (**0.54), and similarly, tubular lumenal macrophages

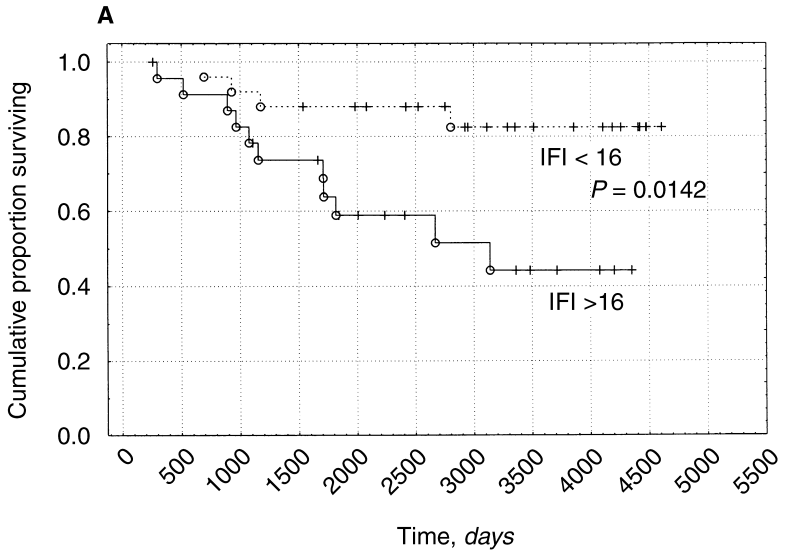
Table 2. Survivals at 3200 days from CRX2 for various indices and morphologic variables

	Score	*N* patients	Proportion surviving	Score	*N* patients	Proportion surviving	*P*
Morphologic Indices[a]							
Biopsy 1							
Glomerular Activity Index 1	≤10	27	0.6566	>10	31	0.7645	NS
Chronicity Index 1	≤2.5	36	0.7979	>2.5	21	0.5247	NS
	≤1	19	0.7782	>4	13	0.5128	NS
Tubulointerstitial Activity Index 1	≤5	28	0.7863	≥8	14	0.4614	0.046
Immunofluorescence Index 1	≤26	25	0.7196	>26	24	0.6539	NS
Biopsy Inflammation 1[a]	≤5.5	32	0.7542	>5.5	26	0.6335	NS
Biopsy Index 1	≤4.2	27	0.7744	>4.2	17	0.5156	0.078
Biopsy 2							
Glomerular Activity Index 2	≤3.5	390	0.7940	>3.5	24	0.4850	0.016
	<3	31	0.8104	>8	8	0.0000	0.0007
Chronicity Index	≤3.8	33	0.7425	>3.8	30	0.6058	NS
Tubulointerstitial Activity Index 2	≤3	22	0.7377	>7	14	0.4726	0.055
Immunofluorescence Index 2	≤16	27	0.8366	>16	22	0.4054	0.005
	≤9	13	0.9231	>24	13	0.3042	0.005
Biopsy Inflammation[a]	≤1.7	38	0.8681	>1.7	22	0.3349	0.00004
	<1	21	0.9444	≥4	9	0.0000	0.00005
Biopsy Index 2	≤2.9	26	0.8106	>2.9	21	0.4381	0.006
	≤2	14	0.8750	>4	12	0.2922	0.002
Individual morphologic variables							
Biopsy 1							
Karyorrhexis/fibrinoid necrosis	0	13	0.5858	>0	45	0.7541	NS
PMNs in glomerular capillaries	0	26	0.7342	>0	32	0.6858	NS
Cellular crescents 1	0	25	0.6866	>0	33	0.7131	NS
Glomerular monocytes 1	0	13	0.7252	>0	45	0.6995	NS
Membranoproliferative features	0	39	0.6712	2+	7	0.8571	NS
Tubular macrophages 1	0	36	0.7623	>0	24	0.5875	NS
Glomerular capillary IF 1	≤11	27	0.6881	>11	23	0.7158	NS
Glomerular mesangial IF 1	≤8	26	0.7673	>8	24	0.6265	NS
Tubulointerstitial IF 1	≤3	28	0.6976	>3	21	0.6793	NS
Vascular IF 1	≤3	29	0.8273	>3	21	0.4987	0.03
IF-IgG 1	≤6	25	0.7112	>6	24	0.6204	NS
IF-C3	≤4	15	0.8750	>6	9	0.3703	0.009
Biopsy 2							
Karyorrhexis/fibrinoid necrosis 2	0	40	0.6851	>0	10	0.2667	0.01
PMNs in glomerular capillaries	0	42	0.6608	>0	8	0.3333	0.13
Cellular crescents 2	0	41	0.6526	>0	9	0.3333	0.01
Glomerular monocytes 2	0	40	0.6759	>0	11	0.3535	0.03
Membranoproliferative features	0	53	0.7084	>0	9	0.4000	0.22
Tubular macrophages 2	0	38	0.7388	>0	12	0.1820	0.007
Glomerular capillary IF 2	≤6.5	25	0.7804	>6.5	24	0.4831	0.08
	<4	10	0.7714	>10	10	0.0000	0.007
Subendothelial deposits-2 IF	<1.5	27	0.6637	>1.5	25	0.6143	0.37
	↓>[b]	23	0.8188	↓<[b]	7	0.3333	0.03
Subepithelial deposits-2 IF	<1	36	0.6702	>1	16	0.5540	0.65
Glomerular mesangial IF 2	≤4	27	0.7787	>4	22	0.4889	0.05
Total glomerular IF	<10.5	25	0.8177	>10.5	24	0.4570	0.02
Tubulointerstial IF 2	≤2.5	29	0.8069	>2.5	20	0.3952	0.01
	0	17	0.7894	>4	7	0.0000	0.01
Vascular IF 2	≤3	26	0.7740	>32	23	0.4785	0.04
	≤1	14	0.6857	>5	7	0.2571	0.03
IF-IgG 2	<5	27	0.8503	≥5	22	0.3630	0.009
IF-IgA2	<1.5	23	0.7446	>1.5	25	0.5459	0.20
IF-IgM2	<1.5	29	0.7140	>1.5	19	0.5342	0.27
IF-total immunoglobulins	<8	29	0.8046	>8	19	0.3762	0.008
IF-C3 2	≥3	16	0.9375	>3	22	0.2759	0.0007
	≤2	19	1.000	>5	12	0.2500	0.00005
IF-C1q2	<4	24	0.8890	>4	25	0.4124	0.0008
Standard indices							
Biopsy 1							
Activity Index 1	≤10	16	0.5113	>10	29	.6813	NS
Chronicity Index 1	≤2	28	0.7134	>2	17	.4867	NS
Biopsy 2							
Activity Index 2	≤4	29	0.7375	>4	22	0.4156	0.03
	≤1	12	0.6268	>6	15	0.2291	0.049
Chronicity Index 2	≤2.5	24	0.6724	>2.5	27	0.5507	NS

Kaplan-Meier estimates of survival from CRX2, with the significance (*P*) of survival differences tested by the log rank test. Where more than one set of values is indicated for a given morphologic index or parameter, the first set of values represents those above and below the mean value, and subsequent sets represent values further removed from the mean. Most individual parameters are divided into 0 and >0, but immunofluorescence scores are cited in terms of the mean value for that parameter. Values with log rank probabilities <0.05 are indicated in boldface.

[a] Biopsy inflammation = *glmono* + *macrlum* + *glpmn* + *pmnlum* + *cresc* + *karyfib* + *intinfl*

[b] Percentage reduction between Bx1 and Bx2



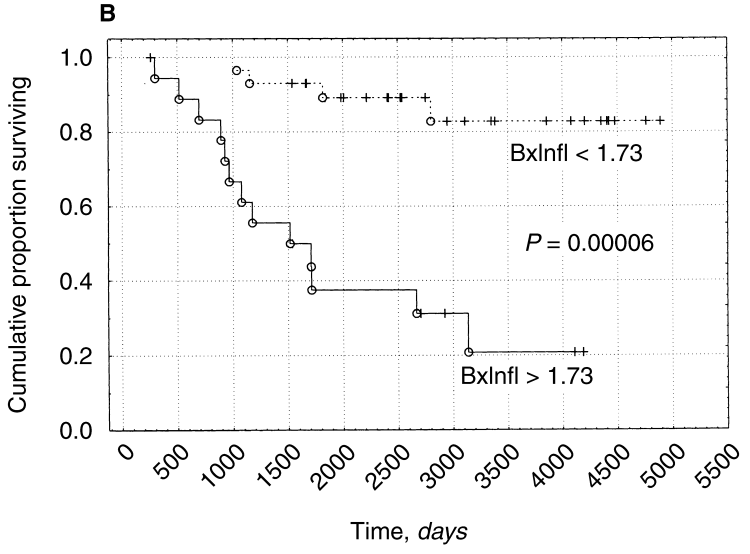


Fig. 1. (A) Immunofluorescence index (IFI): Survival from doubling of serum creatinine (S_{Cr}; CRX2). Results are from biopsy 2. Survival for patients above and below the mean value for IFI = 16. Survivals for patients with IFI > 16 are indicated by a solid line; those with IFI < 16 by a dotted line. Log rank test: $P = 0.01415$. (*B*) Biopsy inflammation, and survival from CRX2. Results are from biopsy 2. Survivals for patients above and below the mean value for BxInfl = 1.73. Survivals for patients with BxInfl > 1.73 are indicated by a solid line, and those with BxInfl ≤1.73 by a dotted line. Log rank test, $P = 0.00006$. The separation between survival curves is greater for BxInfl than for IFI and the probabilities are correspondingly different.

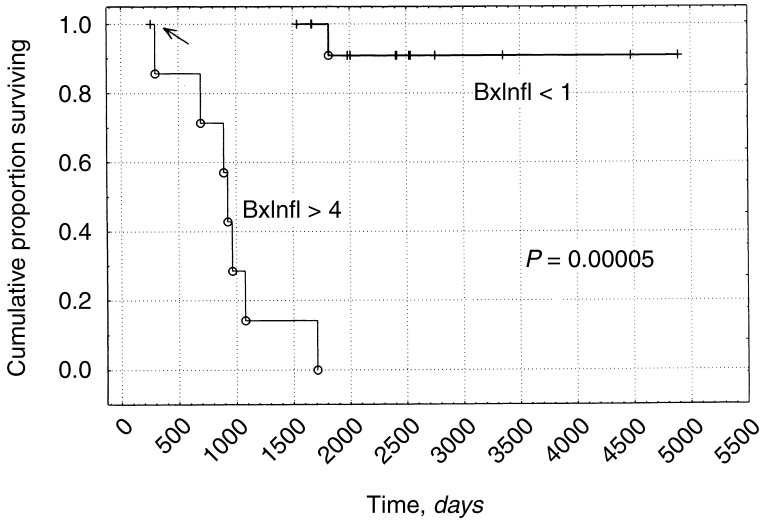


Fig. 2. Biopsy inflammation: Survival from CRX2. Survival curve for Bxinfl <1 is indicated by a thick solid line, and that for BxInfl > 4 by a thinner solid line. Arrow indicates a censored case who died with S_{Cr} = 350 μmol/L, but before qualifying as CRX2.

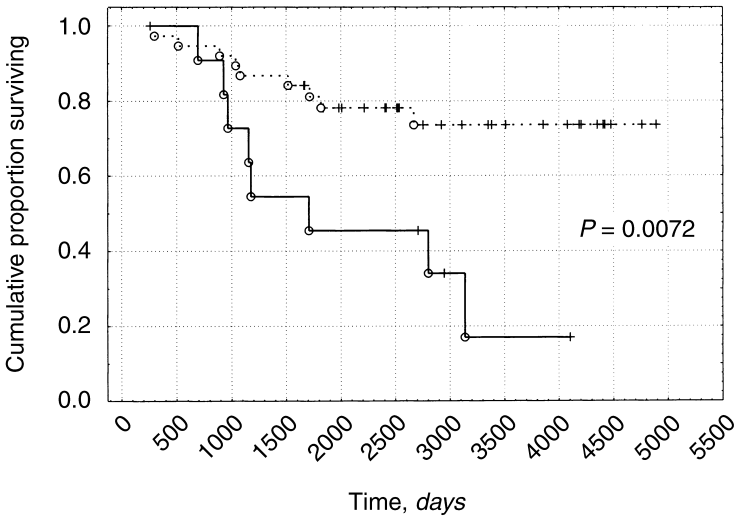


Fig. 3. Tubular lumenal macrophages: Survival from CRX2. Results are from biopsy 2. Survival curve for cases with no tubular lumenal macrophages is indicated by a dotted line, and that for cases with tubular macrophages by a solid line. Log rank test, $P = 0.0072$.

Table 3. Comparison of glomerular and tubular lumenal macrophages (archival material) with immunohistochemical macrophage marker PGM1 in 30 Biopsies

	Glomerular macrophages		Tubular lumenal macrophages	
PGM1$^+$ macrophages	*r*	*P*	*r*	*P*
Glomerular				
Capillary	0.5355[a]	0.002[a]	0.2498	NS
Noncapillary/mesangial	0.3955[a]	0.031[a]	0.1497	NS
Total glomerular	0.5555[a]	0.001[a]	0.1200	NS
Bowman's space	0.2250	NS	0.5482[a]	0.002[a]
Tubular				
Tubular lumenal	0.3361	0.069	0.5246[a]	0.003[a]
Epithelial	0.2709	NS	0.5505[a]	0.002[a]
Total tubular	0.3285	0.076	0.5740[a]	0.001[a]

[a] Significant at $P < 0.05$

correlated well with their PGM1$^+$ counterparts (**0.52). In addition, there were excellent correlations with noncapillary glomerular PGM1$^+$ cells. These latter were often clearly mesangial in location in milder cases (Fig. 4), but with more severe proliferation, their exact location became uninterpretable.

Similarly, there were good correlations between tubular lumenal macrophages and PGM1$^+$ staining in a tubular epithelial location (Fig. 5). The pattern of cytoplasmic staining of these latter cells suggests that the positivity arose because of transformation of existing epithelial cells rather than infiltration by preexisting macrophages. It was seen first in a basal position in cells with columnar appearance and nuclei identical to those of adjacent epithelial cells and gradually expanded to supranuclear regions in a cell less typically epithelial. It only infrequently appeared in discrete cells with rounded contours such as one might see in the tubulitis of allograft rejection. This is not an issue than can be resolved in the present study because the necessary double marker studies necessary for confirmation were not performed. (However, in another study on focal segmental glomerulosclerosis,

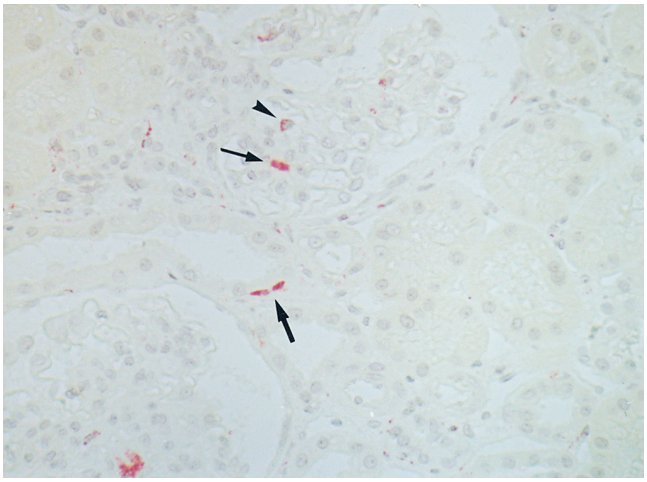


Fig. 4. Glomerular macrophages, PGM1 antiserum: Glomeruli showing lumenal (arrowhead) and mesangial (thin arrow) macrophages. In addition, a nearby tubule shows basolateral staining of two epithelial cells (thick arrow; ×300).

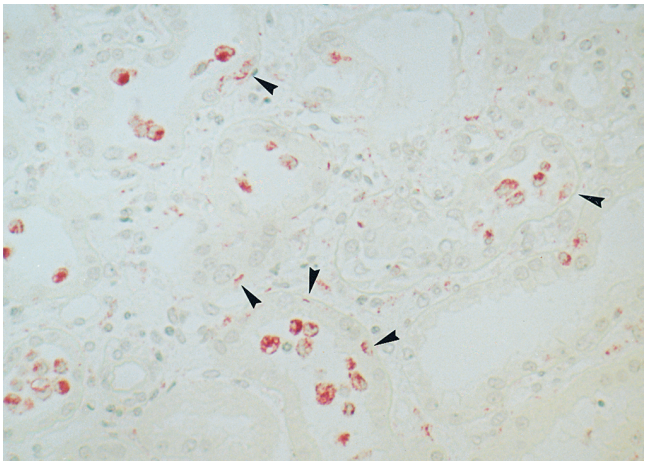


Fig. 5. Tubular macrophages, PGM1 antiserum: In addition to red-staining macrophages free in the tubular lumens, several tubular epithelial cells (arrowheads) show positivity for PGM1, predominantly in a basal position (×300).

Table 4. Correlations between macrophages and serum creatinine (S_{Cr}), proteinuria and renal insufficiency in 30 biopsies stained with PGM1

	S_{Cr}		Proteinuria		Renal insufficiency[b] (16 biopsies at Bx2)	
$PGM1^+$ macrophages	*r*	*P*	*r*	*P*	*r*	*P*
Glomerular						
Capillary	0.4856[a]	0.007[a]	0.6471[a]	0.000	0.6422	0.0073
Noncapillary/mesangial	0.2109	NS	0.3273	0.077	0.8342	0.00006
Total flocculus	0.2161	NS	0.4174[a]	0.022	0.7516	0.00079
Bowman's space	0.5756[a]	0.001	0.2924	NS	0.6158	0.011
Tubular						
Lumenal	0.5485[a]	0.002[a]	0.4994	0.004	0.5605	0.024
Epithelial	0.7385[a]	0.000	0.3259	0.079	0.4761	0.062
Total tubular	0.6770[a]	0.000[a]	0.4509	0.012	0.5325	0.034
Interstitial	0.3412	0.065	0.2555	NS	0.4480	0.082
For comparison:						
Glomerular monocytes[c]	0.3039[a]	0.000[a]	0.4927	0.000	0.3485	0.0048
Tubular lumenal macrophages[c]	0.5089[a]	0.000[a]	0.2636	0.002	0.4408	0.0003

[a] Results significant at $P < 0.05$

[b] Renal insufficiency is defined here as $S_{Cr} > 300$ μmol/L

[c] These results were obtained from observations on routine stains of archival material and include all of the cases in the study at Bx1 and Bx2; in the instance of CRX2 only the results at Bx2 are evaluated.

such doubly positive tubular epithelial cells were identified. In that situation they were infrequent, much less numerous than tubular macrophages.) Finally, there was a strong correlation between the $PGM1^+$ cells in Bowman's space and tubular lumenal macrophages.

Before the beginning of treatment, at Bx1, there were strong negative correlations between $PGM1^+$ glomerular capillary macrophages and IgG (−0.73***), C3 (−0.71**), and C1q (−0.64*). Similar but weaker correlations existed for overall glomerular macrophages. However, after six months of treatment, at Bx2, these negative correlations were effaced. The correlations were mildly positive, with IgG ($r = 0.0087$), C3 ($r = 0.3046$), C1q ($r = 0.2070$), but none were significant.

On the clinical level, there were very strong correlations between glomerular $PGM1^+$ cells and proteinuria on the one hand and tubular $PGM1^+$ cells and S_{Cr} on the other (Table 4). These results are parallel to, but perhaps somewhat better than, the correlations of proteinuria and S_{Cr} and glomerular monocytes and tubular macrophages from archival material indicated at the bottom of Table 4. Similarly, correlations at Bx2 between $PGM1^+$ cells and evolution to renal insufficiency (RI) were excellent, both between glomerular $PGM1^+$ cells and RI (0.75***) and between tubular $PGM1^+$ cells and RI (0.53*). The correlations with RI were, in fact, better than those for glomerular and tubular macrophages for the overall series, and in the instance of glomerular $PGM1^+$ cells, this was significant.

Membranoproliferative features

Cases of membranoproliferative (MPGN) disease were separated from those with other forms of diffuse proliferative disease. They were graded 1+ if there were only occasional double contours in a focal distribution and 2+ if these double contours were diffuse, and the case fairly closely resembled that of MPGN of other causes.

Table 5. Membranoproliferative lesions in diffuse proliferative lupus nephritis

	Biopsy 1		
	MPGN (2+)	Other diffuse proliferative	*P*
N	7 patients	42 patients	
Hypertensive (%)	71.4%	23.8%	0.0059
C3	386 ± 177	539 ± 182	0.0443
DNA	400 ± 419	742 ± 1367	NS
Serum creatinine	212 ± 134	121 ± 55	0.0035
Proteinuria	5.9 ± 4.8	5.1 ± 4.3	NS
Hematuria	42 K ± 39 K	191 K ± 327 K	NS
	Biopsy 2 and subsequent biopies		
	MPGN persists or develops[a]	No MPGN or MPGN disappears	*P*
N	10 patients	39 patients	
ESRD	0.6000	0.1842	0.0069
CRX2	0.6000	0.2821	0.0627
RFlast	335 ± 212	193 ± 170	0.0257

[a] Not included is an additional patient with mixed membranous and proliferative lesions, with MPGN at Bx2, who died shortly after, with $S_{Cr} = 350$ μmol/L, but before technically qualifying as CRX2

Those with full-fledged (2+) MPGN (7 cases) were more likely to be hypertensive at presentation and had much higher S_{Cr} (212 ± 134 vs. 121 ± 55, $P = 0.0035$) and lower C3 (Table 5). Clinically, they did not differ significantly in any other respect, including proteinuria and hematuria. However, they responded well to therapy, and at Bx2, their S_{Cr} did not differ significantly from those without MPGN. Those in whom the MPGN features disappeared had an excellent outcome, with no CRX2, and final renal function of 93 ± 28. In contrast, among three patients in whom MPGN features, even focal, persisted and seven patients in whom MPGN features appeared under therapy (10 patients total), six reached

ESRD (Table 5). In addition, a patient with mixed membranous and proliferative lesions who developed MPGN features at the second biopsy died with marked renal insufficiency (S_{Cr} = 350 μmol/L).

DISCUSSION

Predictive ability of indices

The predictive ability of the AI and CI has been a subject of debate over the years. By way of prologue, it should be remembered that no two series are ever directly comparable with one another, differing greatly in demographic factors such as sex and race, duration of disease prior to treatment, severity of clinical manifestations at presentation, and treatment. As an example, the largest and best-studied group of patients currently extant is that of Austin et al with 166 patients followed prospectively [11]. It contains 29.5% blacks, compared with our series having 11.3% blacks. Although survivals from CRX2 for blacks were the same in the two series (0.50 vs. 0.59), their effect on overall survival is obviously vastly different. It is not surprising, then, that pathologists working with the same morphologic tools should arrive at different conclusions in different series.

With this in mind, some groups have found the AI to be a significant predictor of renal outcome [2, 3, 7, 8, 10, 11, 15, 18]. However, Esdaile et al found that while the AI and heavy subendothelial deposits were significantly correlated with renal failure, they did not add significant predictive value to their clinical model [12, 19]. Others have been even less optimistic about the AI's predictive value, finding no significant correlation between the AI and the outcome [4, 6, 8, 9, 20, 21]. In our own series, neither the standard AI nor the GAI had a significant predictive power at Bx1, but both were predictive at Bx2, although not as strongly as the IFI (Table 2).

The CI, or a variant thereof, has been found to be predictive of outcome by some groups [8, 10, 11, 13, 14, 18, 22]. In 1984, Austin et al found the CI to be a strong predictor [3], but in subsequent publications proposed the alternative combinations of cellular crescents plus interstitial fibrosis [2] and later, high-risk histology [11], both of which had greater predictive power than the CI. Esdaile et al found that the CI was significant but did not add predictive power to their clinical model [12, 23], and later proposed their tubulointerstitial index [24], which also had greater predictive power than the CI. Finally, some groups have found the CI to have weak or no correlations with outcome [4, 6, 9, 15, 20, 21]. In our own case, the Bx1 or Bx2 material was not significant using either the standard or the revised CI. In contrast, the biopsy index, although only marginally significant at Bx1 (P = 0.078), was a strong predictor of CRX2 (and ESRD, data not shown) at Bx2.

In summary, in this series, only the TIAI was predictive at Bx1, and the Biopsy Index marginally so, but at Bx2, the GAI, IFI, and Biopsy Index were strongly predictive of CRX2. The standard NIH AI at Bx2 was also predictive of CRX2, but neither the standard NIH CI nor our revised CLI were predictive.

Predictive ability of individual morphologic variables

Among the individual morphologic variables, the one that has consistently shown the best correlations with outcome in the literature is subendothelial deposits [19, 25–28]. This is particularly true when they persist at second biopsy [19, 25]. Esdaile et al also found that while subendothelial and mesangial deposits at Bx1 were not predictive of outcome, the persistence of subendothelial and mesangial deposits at Bx2 was associated with a poorer survival [19]. Our experience parallels theirs in that these variables were not predictive at Bx1, but glomerular capillary IF, largely reflecting subendothelial deposits, was significant at Bx2 (P = 0.03), as were mesangial deposits (P = 0.05). A direct estimate of subendothelial deposits on IF confirmed that failure to resolve subendothelial deposits between Bx1 and Bx2 was also associated with a poorer survival.

Even more strongly predictive in our series, however, was the persistence of tubulointerstitial deposits on IF, although at Bx1 they had no predictive power (Table 2). Magil et al found tubulointerstitial deposits to be significant [27], but in a later article on the same group of patients with more extended follow-up [15], they were of only marginal significance. As others have found [16], vascular IF was a predictor of CRX2 at both biopsies. Overall though, the IFI at Bx2 was more strongly predictive of CRX2 (P = 0.0026) than any of its individual elements.

Cellular crescents at Bx1 have been found to be predictive in some series [2, 10]. In our series, they were not predictive at Bx1 but were moderately predictive at Bx2. Karyorrhexis with fibrinoid necroses has been found significant by several groups [10, 15, 29], but in our material, although predictive at Bx2, they were not predictive at Bx1. Glomerulosclerosis has also been found to have predictive power by some groups [11, 15, 23, 27, 29] but did not reach statistical significance in our series (discussed later in this article).

Among the tubulointerstitial variables previously described as having prognostic significance at the Bx1 have been tubular atrophy [2, 3, 11, 30], interstitial fibrosis [2, 11], and interstitial inflammation [2, 8, 10, 30, 31]. In our material, these measures, as well as the overall CI, all tended to be worse in those destined to CRX2 at both Bx1 and Bx2, but did not reach significance. However, in this study interstitial inflammation was found to be a pivotal variable, strongly influencing the index into which it was incorporated. The variable, *BxInfl*, which includes interstitial inflammation, was the most discriminatory of

all the variables or indices at Bx2 in separating those later doubling their S_{Cr} from those not doubling it.

Monocytes and macrophages

The role of monocytes and macrophages in the pathology of lupus nephritis has been underappreciated. In this study, glomerular monocytes and tubular lumenal macrophages were the individual variables that correlated best with clinical and outcome parameters. Glomerular monocytes/macrophages have previously been found to be present in high numbers in SLE [15, 32–34] and in our series showed the highest correlation with proteinuria (0.39**) of any morphologic variable at Bx1. A strong correlation between glomerular macrophages and proteinuria has been described in an older study [33] as well as experimentally [35]. Parallel with these observations, urinary levels of monocyte chemotactic and activating factor, responsible for mediating glomerular macrophage infiltration [36–38], have been found to reflect disease activity in lupus nephritis [39].

Although the presence of monocytes on urine cytology has been reported to be associated with renal relapse [40], only one study to date has commented on their prognostic significance in SLE renal biopsies. Magil et al found that the presence of glomerular macrophages, as recognized by nonspecific esterase staining, seemed to confer a protective effect on survival from renal insufficiency [15]. Our results at Bx1 are similar to theirs, although not reaching significance. However, our interpretation is different, in that we also found similar slightly better survivals for patients with karyorrhexis/fibrinoid necrosis, glomerular PMNs, and cellular crescents at Bx1, as opposed to those without. It would be hard to attribute a protective effect to crescents. Rather, these results seem to correspond to the old clinical observation that often it is the most floridly involved cases that respond best to therapy [22]. At Bx2, in the treated patient, the presence of glomerular monocytes had a frankly negative effect on renal survival, with survivals at 10 years of 35.5 versus 75.8% for those without ($P = 0.0371$).

Magil et al did not detail the interrelationships between hyaline deposits, crescents, and glomerular monocytes in their original study [15]. However, in a subsequent study [31], they found inverse correlations between glomerular monocytes and IgG deposits on IF (-0.45) and hyaline deposits (-0.35) by light microscopy. Our results accord with theirs in that we found a correlation of -0.73 ($P = 0.04$) between $PGM1^+$ glomerular capillary macrophages and IgG at Bx1 before treatment, with similar negative correlations for C3 and C1q, but we further found that treatment totally eliminated these correlations at Bx2.

Most attention to macrophages outside the glomerulus has focused specifically on the interstitium, where their numbers have been shown to correlate positively with S_{Cr} and negatively with GFR in SLE [41] and in other disease processes [42–44] and on their role in the progression of renal disease [19, 35, 41]. However, Oda et al have recently studied in detail tubular lumenal cells that they refer to as "giant" intratubular macrophages, which appear from their descriptions and photos to represent the same cells we are describing [44]. In a variety of glomerular conditions (not including SLE), they found that these macrophages correlated well with the S_{Cr} ($r = 0.63$), hematuria, and in the instance of IgA nephropathy, with proteinuria, basically results paralleling those in this study. To our knowledge, we are the first to describe the connection between tubular macrophages and clinical and outcome parameters in SLE, their association with CRX2 and with ESRD (data not shown) being particularly striking.

The origin of the macrophages in tubular lumens is not entirely certain. In other disease conditions, notably focal segmental glomerulosclerosis [46] and nephrangiosclerosis [47], many of the tubular macrophages have been demonstrated to be of glomerular origin, owing to transdifferentiation of podocytes. It seems likely that this is the case here as well, for there is a strong correlation between lumenal macrophages and crescents at Bx1 (0.57***). Likewise, Oda et al found a strong correlation between crescents and intratubular macrophages [44]. However, crescents do not provide the entire explanation, because even at Bx1 there are cases with lumenal macrophages but without crescents, and vice versa, and at Bx2, there is no significant correlation between the two ($R = 0.08$). Bariéty et al have shown convincingly that glomerular podocytes are capable of transdifferentiation into macrophages in noncrescentic lesions such as focal segmental glomerulosclerosis, and it seems probable that this is the more general phenomenon, of which crescents are simply a special example [45, 46].

Macrophages might also conceivably migrate into the tubules from the interstitium, but the studies to give no support to this notion date [45–47], and we saw only very occasional PGM1+ cells with rounded contours in the epithelium consistent with this possibility. In contrast, these studies do support the possibility that macrophages may form by transdifferentiation of tubular epithelial cells. First, in focal segmental glomerulosclerosis, we have observed tubular epithelial cells doubly marked for cytokeratin and PGM1 (unpublished observations). Second, experimentally, tubular epithelial cells have been demonstrated to transdifferentiate into fibroblasts through a series of stages that are morphological similar, that is, initially basolateral then expanding to supranuclear regions, to those we describe for PGM1 staining in tubular epithelium [48]. It will require double marker studies, however, to confirm that this occurs in lupus nephritis.

The relative contributions of glomerular and tubular cells to the intratubular macrophage population remain to be elucidated, but present evidence favors a predominantly glomerular origin (unpublished observations) [45–47].

The role of the tubular lumenal macrophages is not entirely clear. Although experimentally the lipids attached to excreted albumin have a chemotactic effect on macrophages [48], this is not the entire explanation. The association of macrophages with S_{Cr} is higher at both time points than with proteinuria, particularly at Bx2, where the correlation with S_{Cr} is $r = 0.43$, and that with proteinuria is $r = -0.01$. This suggests that tubular macrophages reflect glomerular and tubular injury not directly related to proteinuria. Oda et al have found that the tubular macrophages often adhere to tubular epithelium, via intercellular adhesion molecule-1 (ICAM-1) [44]. Since these macrophages have direct antibody-dependent and -independent cytotoxity in other settings, it seems likely that they are involved in direct tubular damage here as well. Consistent with this view is the observation that at Bx1 there is an excellent correlation between the presence of tubular macrophages and tubular epithelial lesions (0.57****). Regardless of the mechanisms involved, their predictive ability is impressive.

The aim of the original study was to develop a morphologic index that could be applied to routine archival histologic material. The immunohistochemical staining of a subset of biopsies for the macrophage marker PGM1 was designed to validate the archival variables. There was a good correlation between PGM1-labeled cells and simple identification of macrophages on routine stains. However, as was anticipated, the latter was inexact and underestimated their true numbers. The correlations with S_{Cr}, proteinuria, and renal insufficiency were even better using PGM1 than with the identification of macrophages on routine stains. We feel that the addition of stains for macrophage markers to the standard panel of stains in lupus nephritis will add considerably to our ability to interpret the lesions seen, particularly in view of the prognostic significance of tubular and glomerular macrophages.

Membranoproliferative lesions

To our knowledge, no other series has reported an analysis of cases with prominent MPGN features for purposes of prognosis. It seems likely that this has been done but not reported because there was no difference in prognosis between those diffuse proliferative cases with and without MPGN features at first biopsy. This was certainly true in our series, where four of seven patients resolved their MPGN features and finished with normal renal function. In contrast, if MPGN persisted (3 patients) or appeared (7 patients) during treatment, the outlook was poor, with 6 of 10 reaching ESRD (Table 5). (To these patients with diffuse proliferative lesions should be added an additional patient with mixed membranous and proliferative lesions at Bx1, who developed MPGN at Bx2 and died with a S_{Cr} of 350 μmol/L.) Thus, MPGN features are exactly parallel in significance to crescents, which similarly are not predictive at Bx1 but whose persistence at repeat biopsy presages a dim future prognostically [14, 19].

Conclusions

The present Biopsy Index was demonstrated in an earlier article to show greater predictive power and better correlations with current clinical data than the standard NIH Index [1]. However, at the first biopsy, even this index is not adequately predictive of subsequent behavior. The first biopsy should be considered basically as diagnostic and not, except in cases with far advanced lesions, prognostic.

Systematic biopsy at six months has more predictive power by far, in that it provides a measure of the response to therapy. It is better, in fact, than clinical data alone. A dichotomy between an active biopsy and a quiescent clinical picture was quite common in our experience. More than half of the patients who ultimately progressed to CRX2 had S_{Cr} of <100 μmol/L, proteinuria < 1.0 g/24 h, and/or hematuria <5000 rbc/mL at the time of the second biopsy. A future communication, dealing predominantly with the clinical aspects of this study, will demonstrate formally the significant added value of systematic second biopsy over clinical data alone (manuscript in preparation). For the present, it can be said that we feel that a systematic second-look biopsy is an important adjunct in the treatment of lupus nephritis.

The Biopsy Index offers a valuable tool in research endeavors, but would frankly be somewhat unwieldy to use on a routine basis. However, the insights it provides into the importance of persistence of immune deposits and/or significant inflammation at repeat biopsy have immediate practical value in daily practice, on the basis of simple comparison of biopsies without the necessity of formal quantitation. If the pathologist recognizes these features, the clinician can be alerted that despite normal or near normal clinical and laboratory data, the patient is at risk of progression to renal failure.

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